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Genetic Diversity of Nigerian Cashew Germplasm

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1. Introduction

Cashew (*Anacardium occidentale* L) is a tropical tree nut crop that is native to tropical South American countries around Bolivia, Brazil, Peru and West Indies (Nakasone & Paull, 1998; Samal et al., 2003), with the Amazonia forest of Brazil being the centre of origin (Mitchell & Mori, 1987). Recent archeological data from 47-million year old lake sediment in Germany has shown evidence of earlier distribution of cashew in Europe during the Tertiary period, thus, suggesting bio-geographic link between America and Africa continents in the distribution of genus *Anacardium* (Manchester et al., 2007). Since the introduction of the modern cashew into Africa and Asia continents about five centuries ago, the crop has spread widely and these new areas have become the centre of diversity of cashew today. Cashew has now become important commodity export crop in the third world countries like Benin Republic, Cote d'Ivore, Guinea Bissau, Ghana, India, Mozambique, Nigeria, Philippines, Srilanka,Tanzania and Vietnam.

Cashew is a drought resistance and evergreen perennial small tree plant with dense foliage and can grow as high as 15 meters or more. It is a member of *Anacardiaceae* family with about 75 genera and 700 species (Nakasone & Paull, 1998). Other members of the family *Anacardiaceae* include mango and pistachio. Out of the eight species identified in the genus *Anacardium*, only cashew (*occidentale*) is of economic importance because of its edible hypocarp (apple) and nutritious kernel from the drupaceous nut (Fig. 1). Cashew tree is mostly single-stemmed with umbrella-shaped canopy and the flowering is normally preceded by vegetative growth flush at the end of wet season in the southern hemisphere. The flowers comprise of male and hermaphrodite types in varying proportion and are produced at the end of new shoots in the periphery of the tree canopy. And because of the sticky nature of the cashew pollens, the plant tends more to insect pollination, with some low degree of selfing (Northwood, 1966; Masawe, 1994; Feitas & Paxton, 1996, Aliyu, 2008). The kidney shaped nut (drupe) attached to the swollen hypocarp (pseudo-fruit or apple) is the true biological fruit of the cashew tree (Fig. 1). The kernel, eaten as dessert is the most nutritional part of the cashew and important delicacy because of it high protein and low cholesterol fat. It contains about 47% fat and oil, 21% protein and 22% carbohydrates (Fetuga et al. 1975; Nayar, 1998) and 82% of this fat is unsaturated that helps in reducing body cholesterol level. Structurally, the kernel is protected by a hard shell or endocarp and a spongy mesocarp which contains some acidic oil called cashew nut shell liquid (CNSL). The liquid has been reported for a number of potential industrial uses e.g. for brake linings, paints and vanishes etc. The juice derived from the fresh cashew apples contains high content of vitamin C, about three times higher than in citrus and pawpaw. This characteristic informed fresh consumption of cashew apples by many people, but unfortunately, this pseudo-fruit can only be kept afresh for a short time after harvesting because of it high sucrose content that aid rapid degradation (rotten).

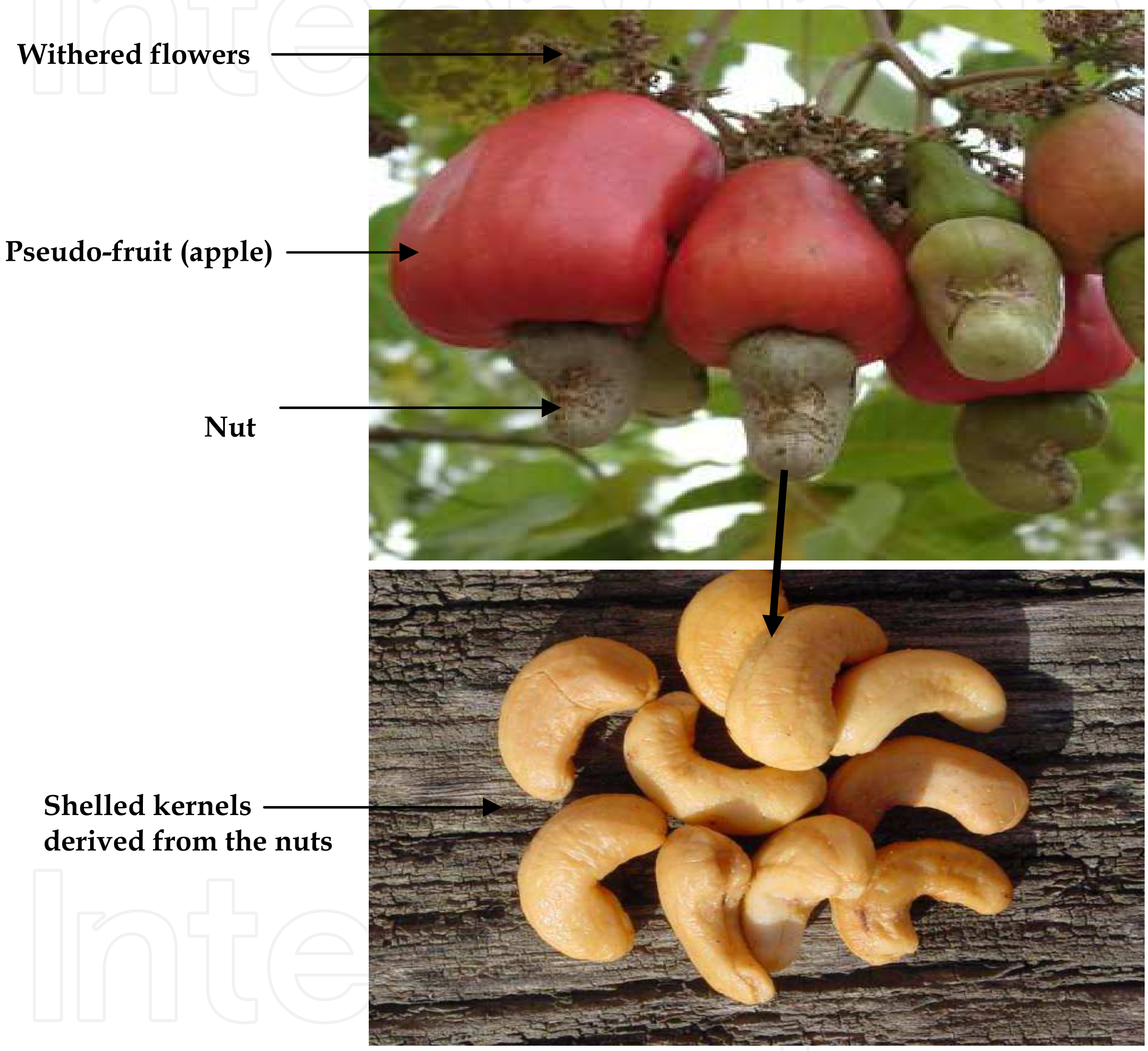


Fig. 1. The most economic products of cashew tree (i.e. hypocarps or apples, the nuts and its derivative kernels after shelling). (Fruit photo from Topper et al., 2001).

2. Cashew economy and production

The world produced about 3.4 million tons of raw cashew nuts in 2009 (FAO, 2011) and about one-third of the world cashew nut production comes from Africa with 50% of the continent exports from Nigeria (FAO, 2011) (Table 1). Cashew nut is one of the important agricultural commodities in Africa and has been contributing to Gross Domestic Product (GDP), National Income (NI) and foreign exchange earnings of many of the African cashew producing nations. For example, cashew is not only one of the export commodity crops in Nigeria but a major source of livelihood for many smallholder farmers especially in the eastern and central parts of Nigeria (Topper et al., 2001). Cashew industry has play important role in the realization of the economic development of many of the African states, and has been one of the veritable platforms for the achievement of the United Nations Millennium Development Goals (MDGs) through economic empowerment of smallholder farmers and rural women, employment generation and small-medium scale industrialization especially in the rural areas (Fig. 2). Because it is currently a predominantly smallholder crop mostly grown as a monocrop though can be intercropped with food crops like cassava, cocoyam, cowpea, ground-nut, maize, pineapple and yam at the early stage of the crop development, cashew farming has been and would continue to provide jobs for teeming rural populace especially the women and youths.

Cashew thrives in a woodland-tall-grass savanna and dry-rainforest ecologies, and such ideal vegetations spread across about thirty (30) states in Nigeria and twenty-seven (27) of these effectively growing cashew as a commodity crop (Figure 3). The producing states are categorized into minor i.e. those with less than 10,000 hectares and major with greater than 10,000 hectares of cashew farms. The minor producing states are mostly from the southwest, south-south and north-eastern states because they combine cashew with other major commodity crops like cocoa, oil-palm, rubber and kola in the south and cereals and pulses in the north. By contrast, states with major plantations are spread across central and southeastern states where emphasis is on cashew production. As a matter of fact, survey of cashew production across the country in 2001 revealed that less than 20% of available cropable lands are under cultivation in most of these states (Topper et al., 2001), which imply prospect for future expansion.

Country/Year	2009	2006	2004	2002	2000	1998	1995	1990	1985	1980	1975	1970	1965	1961
Angola	1667	1590	1307	1139	800	1200	900	1200	1200	1200	1400	1300	1000	1000
Benin	49487	55000	45000	46771	40000	29084	15000	3000	1200	1086	345	627	50	50
Burkina Faso	3168	6141	4904	4364	3732	4015	2500	1074	645	200	N/A	N/A	N/A	N/A
Cote d'Ivoire	246383	235000	140636	104985	63380	39275	39400	6500	3500	600	450	300	400	400
Ghana	35647	34000	25000	9000	7697	8417	1208	480	N/A	N/A	N/A	N/A	N/A	N/A
Guinea-Bissau	64653	95000	96649	86000	72725	64000	29007	30000	13000	3500	2500	2500	2000	2000
Kenya	8381	11349	9332	10031	12500	14531	5000	7000	8500	15000	21600	22200	9000	3000
Madagascar	6072	6700	7289	6349	6500	6500	6000	5300	4000	3400	2900	2400	1900	1600
Mozambique	67846	62821	42988	50177	57894	51700	33423	22524	25000	71100	188000	184000	136000	107000
Nigeria	580761	636000	555000	514000	466000	152000	95000	30000	25000	25000	25000	25000	22000	7000
Senegal	4031	6332	5057	4500	7000	7000	1811	500	N/A	N/A	N/A	N/A	N/A	N/A
Tanzania	79100	77400	92810	55000	121200	93200	63400	17060	32750	41416	115840	107445	76000	50000
Togo	559	700	550	230	320	180	750	587	N/A	N/A	N/A	N/A	N/A	N/A
Total Africa	1151888	1235657	1032655	897746	862998	474736	295474	126858	116960	163218	358035	345772	248350	172050
Total World	3350929	3502184	2900969	2239194	1932142	1249827	1130730	732669	574013	464195	563785	511939	386303	287535
% of World	34.38	35.28	35.60	40.09	44.67	37.98	26.13	17.31	20.38	35.16	63.51	67.54	64.29	59.84

Source: FAO 2011 http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor N/A: Data not available.

Table 1. Cashew production (tons) in some African countries, total production for Africa and the world, and the percentage of Africa's production in the world for the period 1961 to 2009.

Fig. 2. Rural women separating nuts from pseudo-fruits (apple) i.e. postharvest processing (up) and grading of kernels after shelling before packaging (bottom). (Photos from Ghana Cashew Development Project report).

By 1995, the total land area cultivated to cashew in Nigeria was about 40,000 hectares with about 60% by small holders, 20% grow in the "wild", and 20% by the medium-large scale farmers. Currently, cashew cultivation has increased to about 330, 000 hectares (FAO, 2011) (Table 2) and consequently, annual nut production has been on the steady increase too, from 30,000 metric tons in the 1990 to 727,000 metric tons in the 2007 (FAO, 2011) (Table 2). The improvement in production has been attributed largely to increased cultivation and favourable economic policy that encourage more private sector investments.

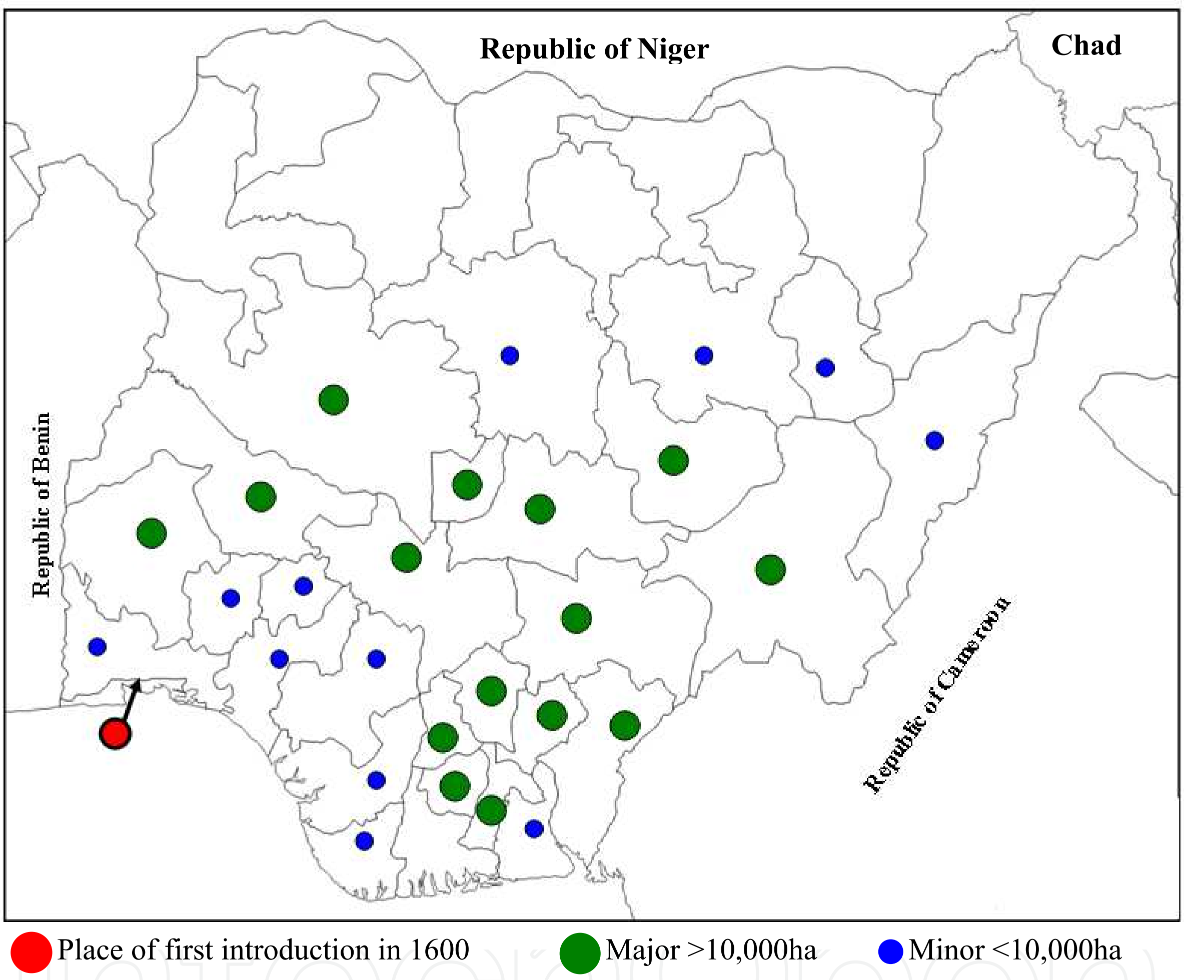


Fig. 3. Point of cashew introduction into Nigeria (Agege, Lagos) in the 16th century and the current production spread across 15 major and 12 minor producing states.

3. Historical perspective of the Nigerian cashew germplasm

Available records showed that cashew was introduced to Africa the same period with India i.e. about 16th century through trade mission by the Portuguese explorers (Johnson, 1973; Mitchell and Mori, 1987). In other word, Nigeria shared similar history with India and the first introductions around 400 years ago were planted in the coastal area around Agege, Lagos, Nigeria (Fig. 3), similar to Goa in India (Archak et al., 2009). Spontaneous planting from this coastal area facilitated its spread to other parts of Lower Niger of the country (Woodroof, 1967; Venkataramah, 1976; Togun, 1977; Ohler, 1979). After the introduction and

Year	Harvested area (ha)	tonnes/ha	Annual Production (tons)
1990	50,000	0.60	30,000
1991	75,000	0.60	45,000
1992	90,000	0.61	55,000
1993	120,000	0.63	75,000
1994	135,000	0.63	85,000
1995	155,000	0.61	95,000
1996	175,000	0.63	110,000
1997	243,000	0.51	125,000
1998	243,020	0.63	152,000
1999	248,000	1.75	417,000
2000	259,000	1.80	466,000
2001	265,000	1.83	485,000
2002	273,000	1.88	514,000
2003	277,000	1.89	524,000
2004	292,000	1.90	555,000
2005	309,000	1.92	594,000
2006	320,000	1.99	636,000
2007	330,000	2.00	660,000
2008	330,000	2.20	727,603
2009	330,000	1.76	580,761

Source: FAO (2011) http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Table 2. Cashew and nut production (land area (ha) yield/ha and annual total output) in Nigeria from 1990 – 2009.

spontaneous spread, cashew trees thrive in the wild for about three centuries (1600-1900s), with no commercial value and exploited mainly for the afforestation and control of gully erosion in most eastern parts of the country with subtropical savannah ecology.

3.1 First and second cashew germplasm introductions

Historically, the development of the Nigerian cashew industry shared history with the political independence of the nation with the emerging regional governments (Eastern, Northern and Western) of self-rule in the 1950s striving for economic independence and development. While the northern region with vast savannah ecology focused on the cultivation of cereals and pulses, the east and west explored tree crops (cocoa, rubber, oil palm and cashew). This period coincided with the development and advancement of cashew nut processing technology by Indians in the 1950s and the demand for raw nuts was on the increase. These historical events led to the establishment of first set of large commercial cashew plantations in Udi and Oghe by the Eastern Nigerian Development Corporation (ENDC) and Iwo, Eruwa and Oke-ogun by Western Nigeria Development Corporation (WNDC) in the period 1953-1960. In addition to sourcing planting materials from the first cashew introductions that had spread across the country (wild and few Agriculture and Forestry Departments), significant percentage of the planting materials (cashew seeds) used for the establishment of these large plantations (ENDC & WNDC) were imported directly from India by the Ministry of Agriculture of these two regional governments, and this constituted the first major cashew genetic resources in the country. Cashew aggressively spread to the other parts of the country from these two regions thereafter and today the crop now grow effectively in twenty-seven (27) out of the thirty-six (36) federal states (Fig. 3) of Nigeria with estimated 330,000 hectares of cultivated cashew land and raw nut outputs of 640,000 metric tons (FAO, 2011). The establishment of large plantations in the country ushered in era of establishment of cashew kernel processing plants for value addition locally. And by the late 1960s to early 1970s, Nigeria was exporting both the raw nuts and processed kernels to India. However, with limited expertise locally, the emerging industry encountered challenges in all areas of the cashew value chain that later led to the enactment of a law in 1971 mandating the Cocoa Research Institute of Nigeria (CRIN) to carry out research and development into production, processing and marketing for the Nigerian cashew industry.

And as a part of the first initiative to improve production, Cocoa Research Institute of Nigeria embarked on its first exploration and germplasm collection from the existing cashew in the wild, farms and plantations (including WNDC) across the country as early as 1973 (Sanwo, 1973), and assembled the collections at the Institute's field gene banks located in Ibadan (Western Nigeria), Uhonmora (Mid-Eastern Nigeria) and Ochaja (North-central Nigeria) thereafter. These cashew collections were later found to be of narrow genetic base (see Akinwale & Esan, 1989; Aliyu & Awopetu, 2007a, 2007b) and step was taken to broadening the genetic pool by introducing more materials from India, Tanzania and Mozambique around 1978 and 1980, which constituted the 2nd germplasm introduction.

3.2 First cashew on-farm evaluation and selection programme

The introduction of germplasm was simultaneously accompanied by on-farm (farmers' fields or plantations) evaluation of some of the selected materials especially at the Western Nigerian Development Corporation (WND)'s plots. The outcome of such preliminary evaluation resulted in the selection of the 25 half-sib progenies (genotypes) with potential high yielding ($\geq$ 1000kg/ha) and were released as improved cashew cultivars tagged G-series by CRIN in the 1980s (see Akinwale & Esan, 1989). These G-varieties have been the main improved cashew materials that were distributed to farmers since 1988. This preliminary research intervention led to a dramatic improvement especially in terms of access to improved planting materials by farmers, which have hitherto sourcing materials from the wild, and laid a strong foundation for a investable cashew industry in the country and neighbouring nations.

3.3 Third cashew germplasm introduction

Introduction of the International Monetary Fund (IMF) structural adjustment programmes (SAP) and adoption of liberalization policy in late 1980s by the then government altered landscape for the cashew trade as well and ushered in increased competition within the African cashew industry with emphasis on quality. This paradigm shift brought into the fore the need for compliance with global quality standard in the competitive markets. Unfortunately, most of the African cashew exports, Nigerian inclusive fell short of the required global grading standards especially in size and quality of the nuts and kernels. The challenge prompted some affluent farmers to introduce new cashew materials (with characteristic bold nuts and high grade kernels) directly from Brazil towards the end of 1980s. In partnership with these farmers, the Cocoa Research Institute of Nigeria collected germplasm accessions from these introductions to expand the National Cashew germplasm base. These Brazilian materials now constitute the third major introduction into Nigerian cashew germplasm. In total, Cocoa Research Institute of Nigeria currently housed about 22 hectares of cashew germplasm field across 6 locations in the country.

4. Challenges for the cashew production in Nigeria

Cashew production in Nigeria like other major producing nations is constrained by low yield and variable nut yields, nut quality and pests and diseases infestation. Incidentally, most of the existing farms were established with open pollinated seeds sourced from the wild and unimproved land races. Expectedly, large percentage of such trees exhibited significant variation in all traits, from growth form, yield to diseases and pests tolerance. For example, cashew trees have been found producing between 0kg and about 45kg nuts per tree with average yield per hectare sometimes between 0.4ton and 1.2tons/ha (Aliyu & Awopetu 2007a). Thus, broadening the genetic base through introduction of new alleles from exotic germplasm (Faenza et al., 1982) and systematic exploitation of heterosis (Masawe, 1994) of the germplasm through a recurrent selection can only be the best solution for tackling yield-related problems in cashew. The Cocoa Research Institute of Nigeria established with a national mandate to address these production challenges has initiated a comprehensive programme for the documentation and evaluation of the existing cashew germplasm in the country with the goal to identify better cultivars that combine higher yield with nut quality (size, colour, etc.). Unfortunately, such characterization and evaluation efforts were biased towards phenotypic and agronomic traits such as nut size, nut weight, sex ratio, colour of apple, size of the fruits, tree canopy, length of panicle and yield performance (Akinwale & Esan 1989; Mneney et al., 2001; Aliyu & Awopetu, 2007a) due to low capacity and limited molecular resources for tree crop research in the country. Although the traditional phenotypic method is useful, more often than not its efficiency could be masked by environmental effect, hence, the need to complement the phenotypic data with molecular method. A preliminary data from the on-going evaluation is summarized in the next section.

5. Update on the genetic diversity of the Nigerian cashew trees

In the last ten years, efforts have been concentrated on the fifty-nine (59) accessions (Tables 3) of Nigerian major cashew germplasm, comprising of three sub-populations (old land races, Indian and Brazilian). These trees were selected because of their reliable passport data and the presence of atleast three replicates for each accession. Available records showed that eleven (11) of these accessions were collected as clonal materials (CC-) from the farmers' fields along the Ochaja-Ankpa axis of the Kogi State (representing north central) in 1987/88. Furthermore, that these materials were probably from the remnants of the first introduction over three centuries ago (Sanwo, 1973; Akinwale & Esan, 1989). The second lot of twenty-three (23) accessions were mainly Indian introductions (CSI-) collected as open pollinated progenies from the Eastern- and Western- Nigeria Development Corporation (ENDC and WNDC) plantations in the 1970s, and were planted in the current locations (CRIN, Ibadan and Ochaja) between 1985 and 1986. And the third set of twenty-five (25) exotic accessions (CSO-) were from cashew materials recently introduced from Brazil by a private farm, Kosoni Ola Farms Limited, Oro, Kwara State and were planted in 1987/1988. These fifty-nine (59) accessions were planted at a spacing of 9.0m x 9.0m, with each accession represented by three entries.

Accession	Source of introduction	Pedigree	Current location
CC01	Unknown	Ochaja area	CRIN, Ibadan
CC02	″	″	″
CC03	″	″	″
CC04	″	″	″
CC05	″	″	″
CC06	″	″	″
CC07	″	″	″
CC08	″	″	″
CC09	″	″	″
CC10	″	″	″
CC11	″	″	″
CSI00	India	ENDC /WNDC	″
CSI01	″	″	″
CSI05	″	″	″
CSI06	″	″	″
CSI07	″	″	″
CSI09	″	″	″
CSI10	″	″	″
CSI11	″	″	″
CSI13	″	″	″
CSI14	″	″	″
CSI18	″	″	″
CSI23	″	″	″
CSI27	″	″	″
CSI30	″	″	″
CSI31	″	″	″
CSI36	″	″	″
CSI51	″	″	″
CSI58	″	″	″
CSI61	″	″	″
CSI62	″	″	″
CSI63	″	″	″
CSI66	″	″	″
CSI67	″	″	″
CSO01	Brazil	KFL Oro.	CRIN, Ibadan &CRIN, Ochaja
CSO02	″	″	″
CSO03	″	″	″
CSO04	″	″	″
CSO05	″	″	″
CSO06	″	″	″
CSO07	″	″	″
CSO08	″	″	″
CSO09	″	″	″
CSO10	″	″	″

CSO11	″	″	″
CSO12	″	″	″
CSO13	″	″	″
CSO14	″	″	″
CSO15	″	″	″
CSO16	″	″	″
CSO17	″	″	″
CSO18	″	″	″
CSO19	″	″	″
CSO20	″	″	″
CSO21	″	″	″
CSO22	″	″	″
CSO23	″	″	″
CSO24	″	″	″
CSO25	″	″	″

ENDC: Eastern Nigerian Development Corporation, WNDC: Western Nigerian Development Corporation, KFL: Kosoni-Ola Farm Limited.

Table 3. List of the fifty-nine cashew accessions and their pedigree analyzed for the yield and yield components variability over a period of ten years (1999-2009).

These cashew trees have attained full maturity (i.e. above 10 years old) at the commencement of the phenotypic evaluation in 1999/2000. Based on the data from previous studies (Masawe, 1994; Azevedo et al., 1998; Aliyu, 2006; Aliyu & Awopetu 2007a), only ten yield-related component characters (whole fruit weight – WWT, individual nut weight – NWT, total nut yield per tree – NYT, kernel weight – KWT, number of hermaphrodite flowers per panicle – HPP, percentage of pollen fertility - PPS, tree canopy size –TCS, days to optimum flowering- DFF, days to optimum fruit maturity - DFM and effective harvesting period (day) – HPD were selected for the phenotypic evaluation. The ten years data (1999-2009) were statistically analyzed using SYSTAT version 13.0 softwares (Systat Software, Inc. USA) and compared thereafter with three years data (Aliyu & Awopetu, 2007a).

Cluster analyses {Eucledian distance ward dendogram and principal component analysis (PCA} from the two studies showed no significant difference between the genetic groupings of the cashew accessions (see Aliyu & Awopetu, 2007a). The data grouped the accessions into five (5) major classes and eight (8) subsets that depict fusion based on source of introduction and/or breeding history (Figs. 4 & 5; Table 4). An overview of the phenotypic (genetic) variability of the accessions showed that the cluster analyses (Figs. 4 & 5) separated the trees into high yielding (clusters I, II & III) and low yielding (clusters IV & V) genotypes. And more than 50% of the accessions fell within the low yielding category. This trend does not only reflect enormous variability that could exist in a typical cashew field, but brought into the fore the level of redundancy in cashew farm in terms of yield performance. To facilitate the efficient utilization of better candidates identified from this evaluation exercise, a short qualitative description of their agronomic importance for each set derived from the cluster analyses (Figs. 4 & 5) is given below.

5.1 Some agronomic values of the Nigerian cashew trees

5.1.1 Very high yielding with moderate quality kernels

A mixture of two (2) Farmers clones and five (5) Indian accessions were the most prolific (producing >3000 nuts/tree/year) of all the fifty-nine accessions studied (Figs. 4 & 5; Table 4). These trees were characterized by heavy fruit clustering on the panicle and have potential to produce >2.5 tons of raw nuts per hectare annually. But the average weight of nuts is about 6.0g and individual nut weight ranging between 4.50 and 8.20g. Derivable kernels from these trees were mixture of W320 and W450 grades with the latter being prominent. The heavy annual production was consistent throughout the study and fruit maturity is mostly in the middle of the season i.e. March. The trees shared prolific fruiting (nut per tree) with accessions that constituted Cluster III (Figs. 4 & 5; Table 4). These cashew accessions are mostly suitable for immediate use as planting materials because of the superior yield characteristic, but little improvement work on the kernel quality is needed to select candidates with stable W320 kernels.

5.1.2 Moderate yielding with high quality kernels

Eleven (11) Brazilian accessions that combine moderate-high yielding, regular production with high quality nuts and kernels were identified from the analysis ((Figs. 4 & 5; Table 4). Weight of individual nut from these trees range between 8.50g and 14.0g, and was characterized by a mixture of W320 and W240 kernels, with the latter being in abundance. Furthermore, these trees were noted for early fruit production and short harvesting time. These materials are better than the G-series that were released and given to farmers since the late 1980s by the Cocoa Research Institute of Nigeria (CRIN) (see Akinwale & Esan, 1989). These eleven superior cashew accessions in addition to those described above (5.1.1) can be clonally propagated for release as improved cultivars to the farmers to meet the short term need and/or undergo further evaluation across different locations through a long term national and/or regional cashew improvement programme. In addition, these new materials can be used for the establishment of polyclonal seed gardens for both research and commercial uses.

5.1.3 Cashew trees prolific fruiting with compact canopy

Nine (9) of the accessions were (see list Table 4 & Fig. 4.) characterized by trees with small and compact canopy, though they are highly prolific in fruiting but the total output per tree were significantly influenced by smallness of its nuts and kernels. The genetic attributes of these nine cashew accessions include small sized trees with compact canopy. These plant materials could be useful for future breeding of cashew cultivars that would be adaptable to high density planting in an effort to improve outputs per unit area.

5.1.4 Cashew trees with low yield and poor agronomic qualities

The characterization exercise also revealed that about 40% of the experimental lots (25 accessions, Table 4; Figs. 4 & 5) were of low yield and poor in agronomic traits. However, because of the large canopy nature of these trees, they are good genetic resources for the afforestation, land reclamation and erosion controlled programmes in the arid regions and areas threatened by gully erosions. The proportion of such trees in the gene pools should be reduced significantly. Further studies are needed to understand the poor correlation between tree size and nut yield in cashew (Masawe et al. 1999; Aliyu & Awopetu, 2007a).

Cluster	Sub-cluster	Accessions in subclusters	No. of Indian accession	No. of Brazilian accession	No. of Farmers Land races	% of total accession (n=59)	% of Indian accession (n=23)	% of Brazilian accession (n=25)	% of Farmers Land races (n=11)
I	Ia.	CC06, CSI62, CSI31, CSI58, CSI66, CC05, CSI36	5	0	2	11.9	21.7	0	18.2
II	IIb.	CSO14, CSO19, CSO20, CSO12, CSO06, CSO05, CSO07, CSO01, CSO02, CSO15, CSO10	0	11	0	18.6	0	44.0	0
III	IIIc.	CSI16, CSO16, CSO14, CSI05, CSI18, CSO03, CSO13, CC11, CSI00	4	4	1	15.3	17.4	16.0	9.1
IV	IVd.	CSO18, CSO11, CC07, CC04, CC09, CSO17	0	3	3	10.2	0	12.0	27.3
	IVe.	CC10, CSI06, CSI30, CSI01, CSI09, CSI61, CSI10, CSI27,	7	0	1	13.6	30.4	0	9.1
	IVf.	CC03, CC01, CC02, CC08	0	0	4	6.8	0	0	36.4
	IVg.	CSI51, CSI67, CSI23, CSI07, CSI13, CSI14, CSI11	7	0	0	11.9	30.4	0	0
V	Vh.	CSO09, CSO08, CSO24, CSO23, CSO25, CSO24, CSO22	0	7	0	11.9	0	28	0

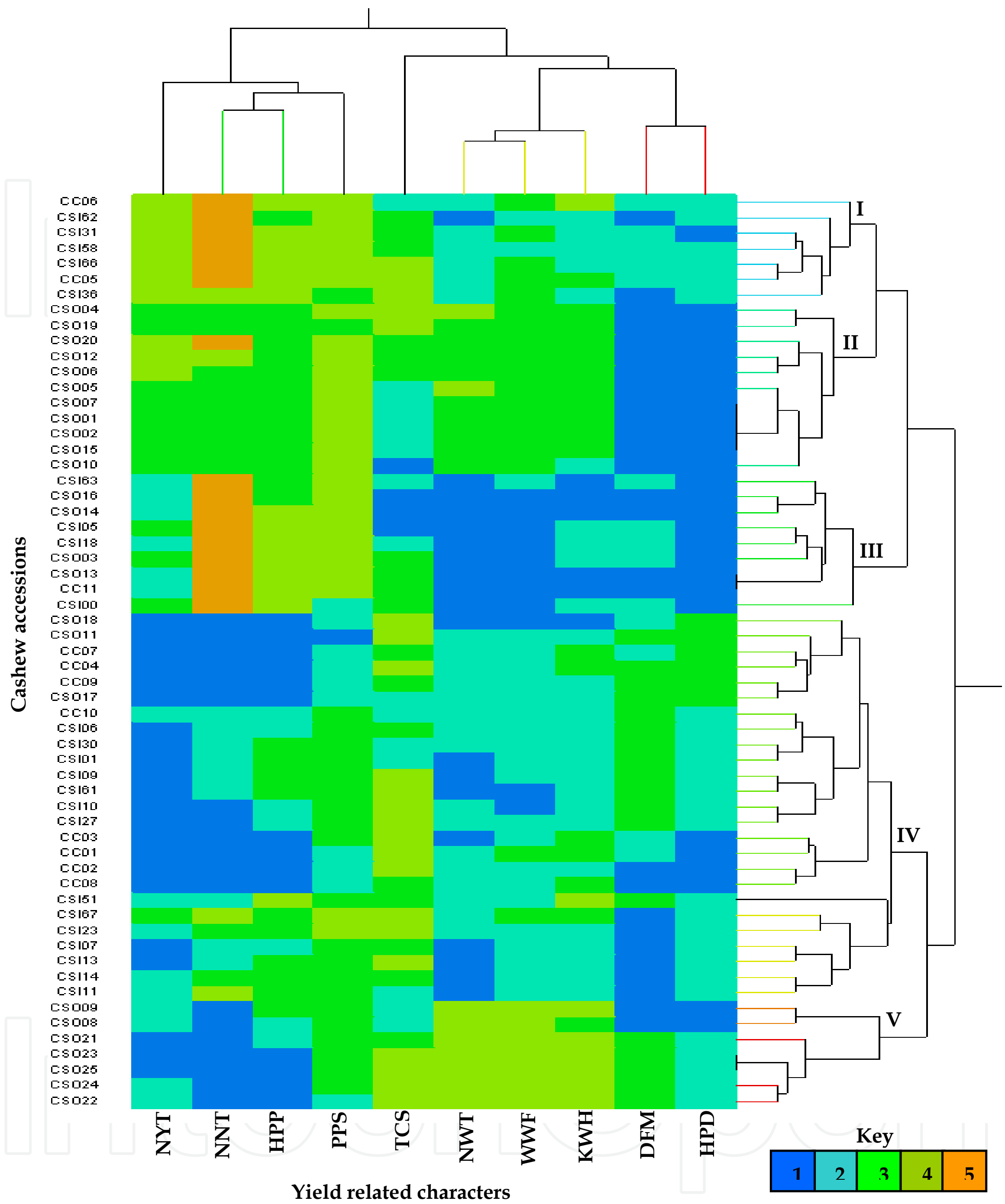
Table 4. Inter- and intra-cluster fusion of the 59 cashew accessions derived from the genetic diversity analysis showing source of origin/or breeding history effect.

5.1.5 Cashew trees with low yield and high quality kernels

Seven (7) accessions that consistently produced fewer but extra fruits (both apple and nut) were identified from the study. They are predominantly Brazilian collections (Table 4 and Figs. 4 & 5) with low yield because these trees rarely produce more than 300-400 fruits per trees. With average nut weight of 16g, these trees can be a good source for the introgression of genes for high grade kernels in cashew breeding programme. Other attributes of these five accessions include high volume of apple juice yield with low astringency. This category of cashew trees are often referred to as Jumbo varieties by farmers and are inadvertently collected as planting materials from unapproved sources. Unfortunately, recent study on nut size and number trade-off in cashew (Aliyu & Awopetu, 2011) has shown poor yield in this category of cashew trees, thereby corroborating this result. Hence, such materials are not suitable for the establishment of investable cashew farms, but could be used as a good source of genetic resources for research and developments of better varieties/cultivars.

6. Recurrent selection strategy for the development of hybrid cashew

Following the conclusion of the evaluation exercise and grouping of the entries into their respective agronomic groups, the Institute is embarking on a long term recurrent selection strategy for the development of improved cashew varieties. Apart from multilocational evaluation of most of the superior materials identified in the characterization exercise, the breeding plan included exploitation of heterosis between the highly prolific, but small fruits



Legends for the characters and key.

NYT:- Total nut yield per tree per year (kg) (1-Low, 2-Moderate, 3-High, 4-Very High); NNT:- Nuts per tree/year (1-Low, 2-Moderate, 3-High, 4-Very High, 5- Extra-super high); HPP:- Hermaphrodite flowers per panicle (1- Low, 2-Moderate, 3-High, 4-Very High); PPS:- Pollen grain fertility (%) (1- Low, 2-Moderate, 3-High, 4-Very High); TCS:- Tree canopy size (m²) (1- Compact, 2- Moderate, 3- Large open, 4- Spread with extensive branches); NWT:- Nut weight (g) (1- Small, 2- Medium, 3- Large, 4- Extra large); WWF:- Whole fruit weight (g) (1- Small, 2- Medium, 3- Large, 4- Extra large);KWH:– kernel weight (g) (1- W450, 2-W320, 3-W240, 4-W180); DFM:- Days to optimum fruit maturity (1- Early, 2-Mid-season, 3- Late); HPD:- Harvesting period (1-Short, 2- Intermediate, 3-Prolong).

Fig. 4. Phenotypic variability of the between fifty-nine cashew germplasm accessions for the nine yield related components as obtained from the ten (10) years data (1999-2009).

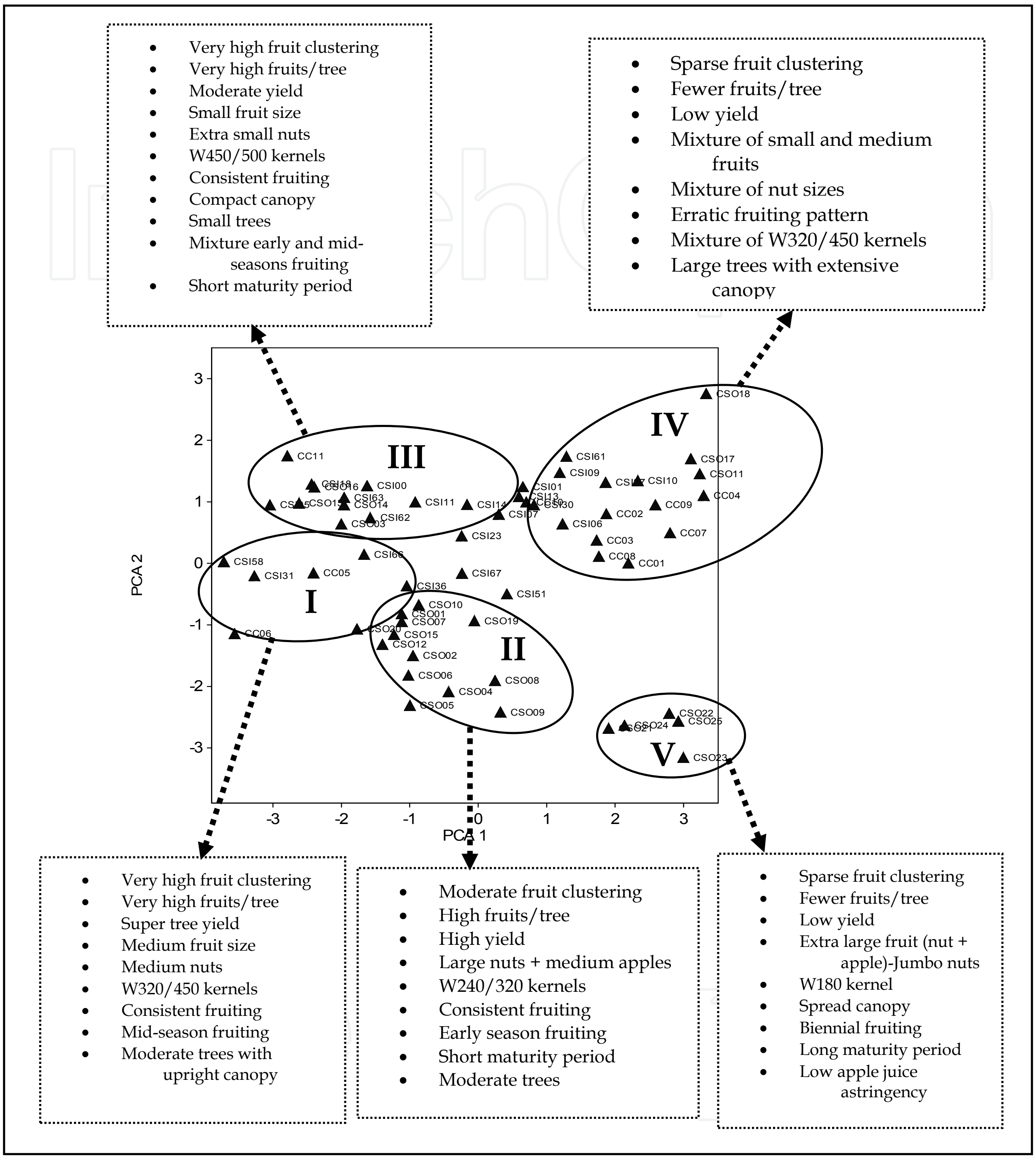


Fig. 5. Principal component analysis (PCA) of the fifty-nine cashew accessions showing five clusters and their respective agronomic characteristics.

and very low productive and extra large fruit trees through controlled hybridization and *in vitro* embryo culture, where necessary. Less impediment is expected from the hybridization exercise because of the high cross-compatibility in cashew (Aliyu, 2007, 2008), albeit, that the environmental conditions are favourable.

7. Application of protein-isoenzme electrophoresis as a useful tool for cashew characterization

In the absence of a well equipped laboratory with recent markers such as Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSRs), protein-isozyme sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has proven as a reliable alternative for the characterization of cashew germplasm. The PAGE analysis differentiated the accessions into six overlapping subclasses similar to the results of morphological characterization i.e. corroborating groupings based on phenotypic attributes of the trees (see Aliyu & Awopetu, 2007b). For example, out of fifteen (15) accessions of the Brazilian origin grouped together as a cluster I at 43.0% linkage distance by PAGE analysis (see Aliyu & Awopetu, 2007b), eleven (11) were clustered together as Cluster II in the 10 year phenotypic data, suggesting genetic congruity between phenotypic attributes and breeding history/or and source of origin of these cashew plants. Similarly, five accessions CSO24, CSO25, CSO22, CSO23 and CSO21 that are morphologically and agronomically similar for their extra-large (jumbo) fruits (nut and apple), late and irregular flowering and fruiting characteristics, and spreading and extensive branching pattern, clustered together on the PAGE analysis because they shared common isoenzymes and probably reinforcing theory of source of origin and/or increased nuclear DNA content as the underline genetic factor for the grouping. Beside the general characteristics, isoenzyme study further differentiates the accessions within a sub-cluster on the basis of apple skin pigmentation into red, yellow and orange. Similar to the results of the ongoing molecular (SSR) analysis, the protein-isozyme study equally revealed moderate genetic base for the Nigerian cashew germplasm. The result of the PAGE analysis (Aliyu & Awopetu, 2007b) has demonstrated that this biochemical method can be used as a reliable and effective alternative characterization tool in the assessment of genetic relationships and grouping into morphotypes in cashew.

8. Microsatellite analysis reveals genetic redundancy in Nigerian germplasm

In collaboration with the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, one hundred and eighty-seven (187) cashew accessions from different populations are being evaluated using 10 simple sequence repeats (SSR) markers (Croxford et al., 2006) (Table 5). An overview of the phenogram derived from the molecular data showed that eight (8) of the ten (10) microsatellite loci except, mAoR2 and mAoR47 were polymorphic (Fig. 6) and could be efficiently used for characterization and management of cashew germplasm. Preliminary data from the study showed significant level of redundancy (homogenous group) (Fig. 6) within the Nigerian cashew germplasm and the need to develop a core collection to reduce the cost of management of cashew germplasm. The data also depicts narrow genetic base of the Nigerian cashew germplasm. Apparently, future efforts at enhancing genetic base of Nigeria cashew germplasm should focus on collaborations and extensive linkages with relevant research institutions from Asia and South America.

9. *In vitro* embryo culture in cashew

Embryos from immature nuts of cashew have been successfully cultured *in vitro* at the Cocoa Research Institute of Nigeria, to establish a routine protocol for the regeneration of plantlets from crosses between distant parents (genotypes). In the trial, 2-, 4-, 6- and 8-weeks

Locus	Primer sequence (5′- 3′)	Repeat motif	T_a (°C)	Allelic size range (bp)
mAoR2	F: GGCCATGGGAAACAACAA R: GGAAGGGCATTATGGGTAAG	$(CA)_{10}(TA)_6$	58.2	366-375
mAoR3[a]	F: CAGAACCGTCACTCCACTCC R: ATCCAGACGAAGAAGCGATG	$(AC)_{12}(AAAAT)_2$	60.3	241-247
mAoR6[c]	F: CAAAACTAGCCGGAATCTAGC R: CCCCATCAAACCCTTATGAC	$(AT)_5(GT)_{12}$	58.2	143-157
mAoR7[b]	F: AACCTTCACTCCTCTGAAGC R: GTGAATCCAAAGCGTGTG	$(AT)_2(GT)_5AT(GT)_5$	58.2	178-181
mAoR11[c]	F: ATCCAACAGCCACAATCCTC R: CTTACAGCCCCAAACTCTCG	$(AT)_3(AC)_{16}$	60.3	234-236
mAoR17[b]	F: GCAATGTGCAGACATGGTTC R: GGTTTCGCATGGAAGAAGAG	$(GA)_{24}$	56.1	124-159
mAoR42[c]	F: ACTGTCACGTCAATGGCATC R: GCGAAGGTCAAAGAGCAGTC	$(CAT)_9TAT(CTT)_7$	60.3	197-206
mAoR47	F: AAGAGCTGCGACCAATGTTT R: CTTGAACTTGACACTTCATCCA	$(TAAA)_2(TA)_7(AAT)_5$	58.2	161-173
mAoR48[a]	F: CAGCGAGTGGCTTACGAAAT R: GACCATGGGCTTGATACGTC	$(GAA)_6(GA)_3$	58.2	172-178
mAoR52	F: GCTATGACCCTTGGGAACTC R: GTGACACAACCAAAACCACA	$(GT)_{16}(TA)_2$	58.2	191-203

Table 5. List of ten (10) microsatellite markers with optimal annealing temperature (T_a) and allelic size ranges (Croxford et al. 2006) used for the molecular analysis of the genetic diversity in 187 accessions of cashew.

old embryos were evaluated on different media compositions ranging from pure Murashige and Skoog (MS) agar medium (Murashige & Skoog, 1962) to modified MS medium supplemented with 1 mM each of naphthaleneacetic acid (NAA), benzyladenine (BA) and gibberellic acid (GA_3). The results showed that age of the embryo significantly influenced the rates of response and survival of the plantlets, with older embryos i.e. 6 weeks old and above performed better (Table 6) (Aliyu & Awopetu, 2005). Among the media composition tested, only modified MS medium supplemented with 1 mM of gibberellic acid (MS+GA_3)

Age of embryos (WAPo)[a]	MS[b]	MS+GA_3[c]	MS+NAA[d]	MS+BA[e]
2	0.00 (0.00%)	5.91 (9.85%)	0.00 (0.00%)	0.00 (0.00%)
4	12.99 (21.65%)	19.71 (32.85%)	14.79 (24.65%)	15.69 (26.15%)
6	37.20 (62.00%)	41.31 (68.85%)	36.09 (60.15%)	36.81 (61.35%)
8	45.69 (76.15%)	48.69 (81.15%)	43.20 (72.00%)	42.30 (70.50%)
DMRT ($\bar{x}$)	7.99b	9.63a	7.88b	7.85b

Percentage in parenthesis. LSD 0.05 = 0.332. DMRT: Duncan multiple range test.
a: WAPo: Week after pollination
b: Murashige and Skoog (MS) medium
c: MS medium + gibberellic acid
d: MS medium + naphthaleneacetic acid
e: MS medium + benzyladenine.

Table 6. Effect of age on the growth and development of cashew embryos in different *in vitro* culture media compositions.

supported growth of young embryos of 2 weeks old. In order words, factors such as medium composition, age of embryo and sometimes the genotype influence success rate of *in vitro* culture of cashew embryos. Photoperiod, temperature, source of explants, browning, and contamination are other known factors capable of affecting *in vitro* propagation of cashew (Jha, 1988; Das et al., 1996). The study deed showed that older cashew embryos seem to be autonomous of growth regulators i.e. inclusion of these synthetic plant hormones into media composition only became critical for very young explants.

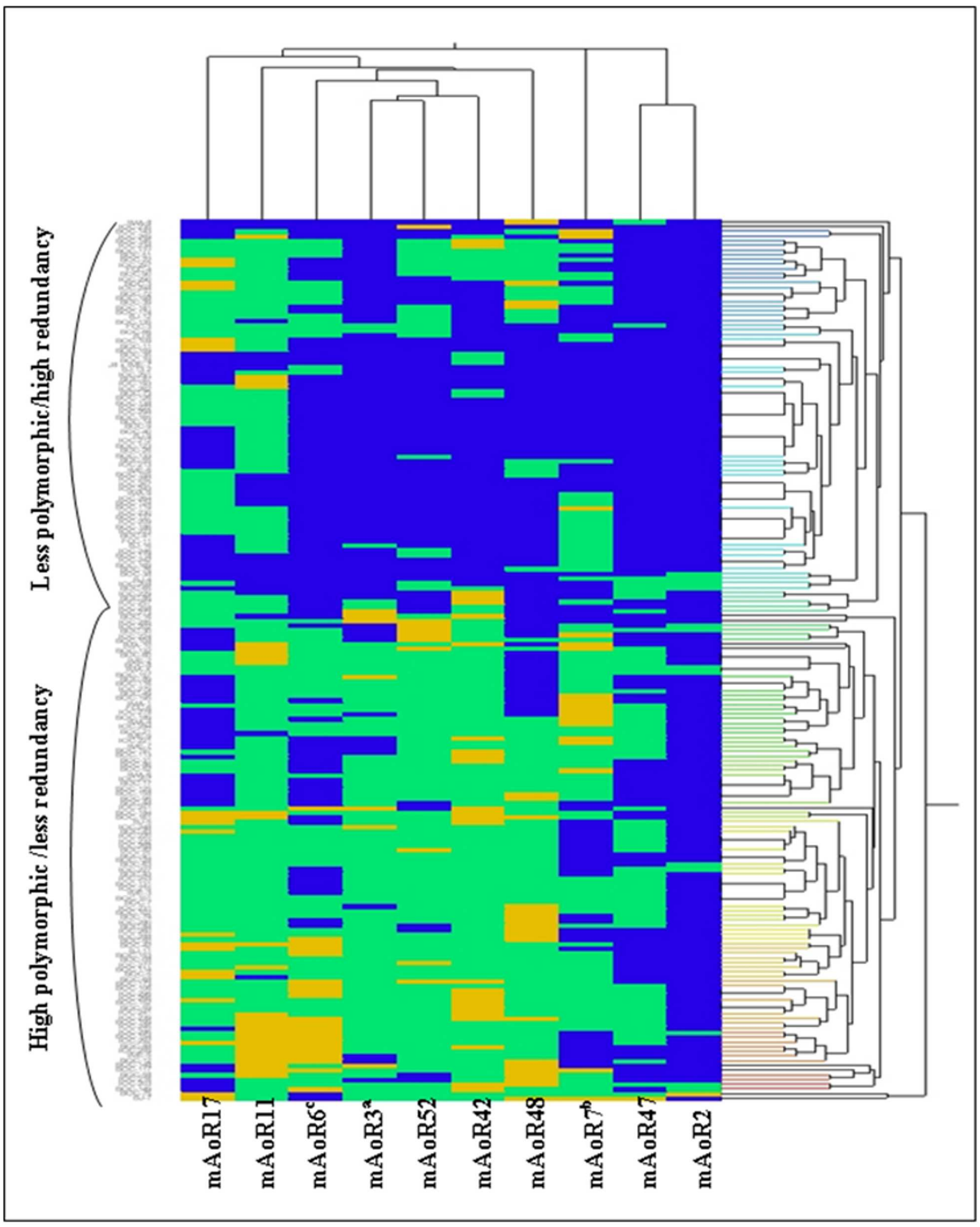


Fig. 6. An overview of the genetic diversity of 187 Nigerian cashew accessions from different populations derived from molecular analysis of 10 microsatellite marker loci.

10. Cashew cytology and cytometry

10.1 Relative nuclear DNA content and genome size in cashew

Information on genome size and ploidy needed for basic molecular breeding of this important commodity is rare. Recent flow cytometric analysis of fifty-four (54) cashew accessions from the Nigerian germplasm was carried out to determine the relative genome size, intraspecific variation and ploidy status of the species using *Solanum lycopersicum* cv. Stupicke as an internal standard reference. And because of the dearth of literatures on application of flow cytometry to cashew or its relatives, the study was preceded by the protocol optimization for the buffer system, sample size, internal reference standard and incubation time for isolated nuclei before analysis (Aliyu, 2011under review). From the analysis of the nuclear suspension in terms of fluorescence intensity, background yield (%), nuclear yield (nuclei s^{-1} mg^{-1}) and coefficient of variation (%) of *G0/G1* peak, the cashew plant showed preference for Otto's buffer, leaf sample size of about 70mg and maximum of 20 mins incubation period. *S. lycospersicum* cv. Stupicke and *Glycine max* cv. Polanka are the ideal internal reference standards for the genome size determination in cashew (Fig. 7a). The average relative nuclear DNA content in cashew is small with relative genome size of 2C = 1.01 pg i.e. about 988Mbp (1C = 490Mbp) recorded for the analyzed accessions (Aliyu, 2010). The relative nuclear DNA contents ranged from smallest (0.903 pg) to largest (1.285 pg) i.e. about 1.36 – fold range. Slight variation for relative genome size was recorded between the accessions (Fig. 7b) and it would be too hasty to conclude that such observation is an

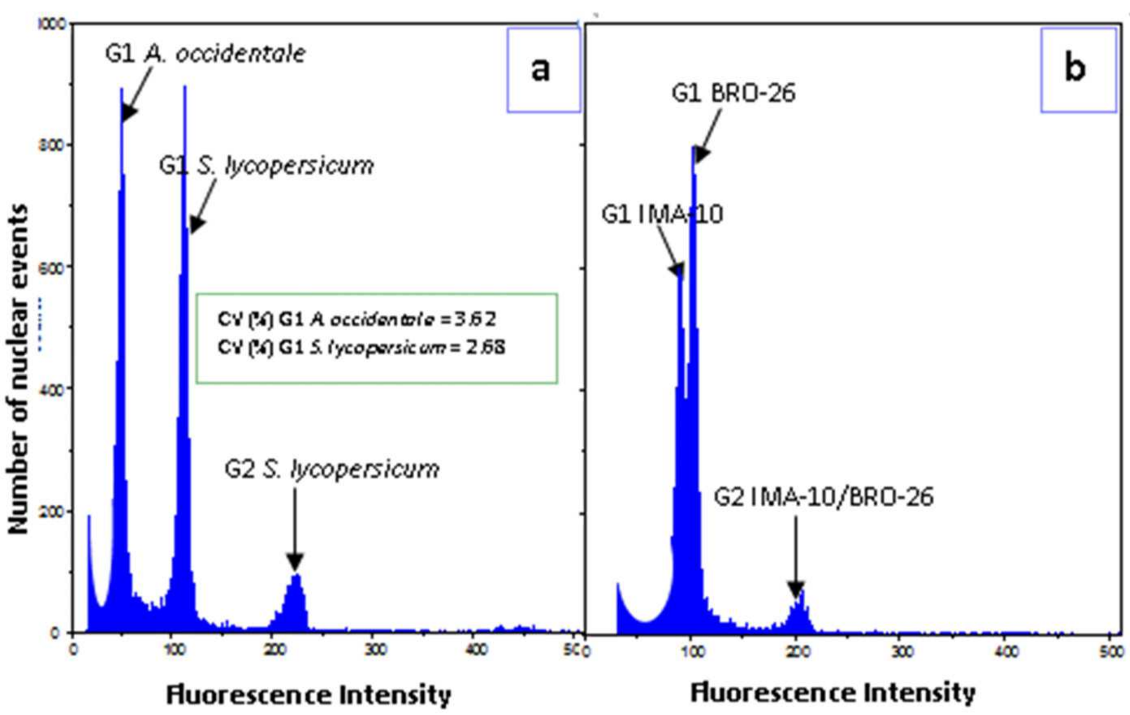


Fig. 7. (a): Flow cytometric histogram showing relative DNA content between G1 nuclei of cashew (*A. occidentale*) and tomato (*S. lycopersicum*) (tomato as internal reference standard). (b): Histogram showing suspected intraspecific variation in relative DNA content between two accessions (IMA-10 and BRO-26) of cashew (*A. occidentale*) with different phenotypic characteristics (see Aliyu, 2010).

intraspecific variation. However, all the sampled accessions showed consistent histogram peak position throughout the measurement, suggesting stability of the genome ploidy and the likelihood that the cashew, *A. occidentale* are predominantly diploids. Slight variation in genome size distribution tends to correlate with the history and/or source of origin/introduction and ecological adaptation of the accessions (Aliyu, 2010). Further studies of the cashew genome size more importantly with a more sensitive dye like Propidium Iodide (PI) may provide clearer information on the absolute genome size, likelihood of intraspecific variation and genome stability of this tropical tree species.

10.2 Chromosome counts in cashew

Aceto-orcein squashing of meristematic tissues (root tips and flower buds) of some genotypes has been analyzed for the number, structure and behavior of chromosome in cashew. Although there are divergent opinions on the ploidy status of the *occidentale* species, the study revealed that most genotypes had 42 chromosomes (Aliyu and Awopetu, 2007c) and could be tentatively described as $2x = 42$ for a diploid species. And that cashew karyotypes are usually symmetric and less divergent, with mainly metacentric and submetacentrics pairs (Aliyu and Awopetu, 2007c). There were similarity in the morphology, number and behavior of the chromosomes among genotypes from different sources of introduction, which could imply common progenitors and slow mutation rate (chromosomal) among the existing *occidentale*. Another possible explanation for the chromosomal congruity can be drawn from the outcrossing nature of the plants, thereby enhances exchange of DNA materials between close and distant sympatric relatives and overlap in phenotypic features between lineages. Such phenotypic similarity between genotypes permits cross-compatibility (free gene exchange) with improved adaptation.

11. Conclusions and future outlook

11.1 West Africa regional cashew improvement programme

With the establishment of African cashew alliance (ACA), a body responsible for the improvement in the value chain in the African cashew industry in 2006, the investments and production of cashew in West Africa region has been on the steady increase. West African countries like Benin, Burkina Fasso, Cote d'Ivore, Gambia, Ghana, Guinea-Bissau and Senegal, which have hitherto not been active in cashew production, are now actively investing in the industry. Unfortunately, as indicated in the report of Sustainable Tree Crops Programmes in 2001, collectively the region lacks expertise and improved varieties to support a sustainable cashew industry. Hence, there is the need to evolve a well funded and coordinated regional cashew improvement programme to aggressively develop improved planting materials for the farmers to boost production and improve the livelihood of the rural households. To achieve this, there is the need to carry out extensive survey of cashew growing areas, document the existing genetic resources and evaluate (characterization) these materials for useful agronomic and yield traits using a set of standardized descriptors across the region. With massive production of cashew in Nigeria (highest in Africa), extensive germplasm, and about four decades of research in cashew by the Cocoa Research Institute of Nigeria (CRIN) and existing research linkages with other sister institutes in Ghana and Cote D'Ivore, Nigeria is better positioned to serve as a platform for such regional effort. And for smooth implementation and meaningful impact on the rural people, such initiative should be in partnership with non-governmental organizations (NGOs) like Sustainable Tree Crops Programme (STCP), Common Funds for Commodities (CFC), African Cashew Alliance (ACA) and other relevant stakeholders in the industry. Similar effort has been used to reposition cashew industry in the East Africa in the recent past.

In addition, the proposed regional programmes should include, establishment of polyconal seed gardens across cashew growing communities for easy access to improved planting materials, development of hybrid cashew that will out-perform the existing cultivars and routine training and capacity building for all the stakeholders to boost production, enhance value addition and above all, improve standard of living of cashew small holders.

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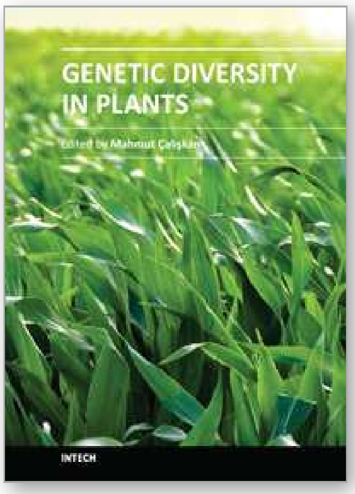

Genetic Diversity in Plants

Edited by Prof. Mahmut Caliskan

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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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